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TESTING PROCEDURES

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TESTING PROCEDURE

T. P. No. 01 Name TEMPERATURE MEASUREMENT

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I. Materials

Metal-stem thermometer or other non-breakable temperature measuring device in graduations of not more than 2°F. Accuracy must be not more than ±2°F.

Sharp metal object or drill to make holes in frozen product.

II. Procedure

For liquids and non-frozen product, thermometer should be inserted at least half-way into material to be tested. For product, the shoulder or other bulky area should be tested.

Fro frozen products, a hole should be bored into a thick section, and the temperature measuring device inserted.

Thermometers should be standardized as needed, or at least on a daily basis by immersing in an ice-water bath (32°F) or boiling water (212°F), and calibrating against an accurate mercury thermometer.

TEN BY:	TECHNICAL DIRECTOR:	QUALITY CONTROL DIRECTOR:	V.P. TECHNICAL SERVICES:

CASTLE & COOKE FOODS

TESTING PROCEDURE

GLAZE DETERMINATION OF IN-SHELL CRAB PRODUCTS
NET CONTENTS OF FROZEN SEAFOODS

T. P. No. 02 Name

Date March, 1980 Issue Draft Page 1 of 1

I. Official Methods of Analysis of the Association of Official Analyticel Chemists, 12th Edition, 1975.

Set scale on firm support and level. Adjust 0 load indicator or rest point and check sensitivity. Scale should be "Exact Weight" balance - type scale, accurate to 0.10 07 (or equivalent).

A. Glazed Seafoods:

Remove package from low temperature storage, open immediately, and place contents under gentle spray of cold H_2O (temperature less than 45°F). Agitate carefully so product is not broken. Spray until all ice glaze that can be seen or felt is removed. Transfer product to circular number 8 sieve, 8" (20 cm) diameter for 2 lb (0.9 kg) and 12" (30 cm) for 2 lb. Without shifting product, incline sieve at angle of 17-20° to facilitate drainage and drain exactly two minutes (stop watch). Immediately transfer product to tared pan (B) and weigh (A). Weight product = A - B.

Percent glaze is weight of ice removed divided by initial weight of product prior to de-glazing.

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TESTING PROCEDURE

T. P. No. 03 Name "BLUEING" REACTION EVALUATION

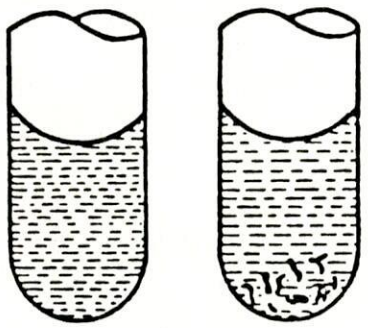
Date March, 1980 Issue Draft Page 1 of 1

I. Procedure

Remove three sections from basket either after chill or freeze operation. If frozen, allow to thaw, and let stand at room temperature for 45 minutes uncovered. Observe for blue or black discoloration in blood sinuses, coagulum, or thicker portions of meat.

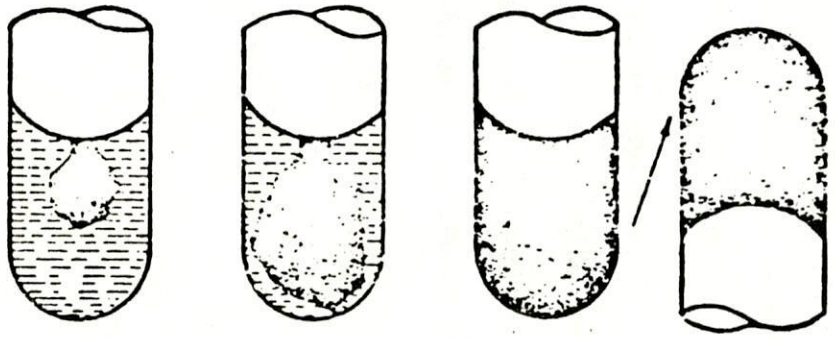
TTEN BY:	TECHNICAL DIRECTOR:	QUALITY CONTROL DIRECTOR:	V.P. TECHNICAL SERVICES:

TYPES OF COAGULASE TEST REACTIONS



NEGATIVE

[no organized clots]



POSITIVE

[organized clots]

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TESTING PROCEDURE

T. P. No. 4 Name SALINITY MEASUREMENT OF CONCENTRATED BRINE

Date April, 1981 Issue Draft Page 1 of 1

I. MATERIALS

A. Salometer - 0 to 100% saturation in 1% division.

B. Non-breakable container with handle (at least 250 mm deep).

C. Low-temperature thermometer in 1°F graduations.

II. PROCEDURE

A. Fill container with brine to be tested by scooping. Float salometer in solution and simultaneously take temperature of solution. Record salometer reading and adjust according to the temperature correction factor (see salometer correction chart). The salometer should never be floated in the brine tank itself due to the potential danger of glass contamination.

B. See Sodium Chloride Brine Tables on next page.

WRITTEN BY:	TECHNICAL DIRECTOR:	QUALITY CONTROL DIRECTOR:	V.P. TECHNICAL SERVICES:

SODIUM CHLORIDE BRINE TABLES for Brine at 60°F.

SALOMETER DEGREES	SPECIFIC GRAVITY	BAUME DEGREES	PER CENT SODIUM CHLORIDE BY WT.	POUNDS PER GALLON OF BRINE		GRAMS PER LITER OF BRINE		FREEZING POINT DEG. F.*
				NaCl	WATER	NaCl	WATER	
0	1.000	0.0	.000	.000	8.328	.0	998.0	+32.0
2	1.004	0.6	.528	.044	8.318	5.28	996.5	+31.5
4	1.007	1.1	1.056	.089	8.297	10.61	994.0	+31.1
6	1.011	1.6	1.584	.133	8.287	15.97	992.8	+30.5
8	1.015	2.1	2.112	.178	8.275	21.38	991.3	+30.0
10	1.019	2.7	2.640	.224	8.262	26.84	989.9	+29.3
12	1.023	3.3	3.167	.270	8.250	32.35	988.3	+28.8
14	1.026	3.7	3.695	.316	8.229	37.86	985.9	+28.2
16	1.030	4.2	4.223	.362	8.216	43.43	984.5	+27.6
18	1.034	4.8	4.751	.409	8.202	49.03	982.7	+27.0
20	1.038	5.3	5.279	.456	8.188	54.65	981.0	+26.4
22	1.042	5.8	5.807	.503	8.175	60.32	979.4	+25.7
24	1.046	6.4	6.335	.552	8.159	66.13	977.4	+25.1
26	1.050	6.9	6.863	.600	8.144	71.88	975.7	+24.4
28	1.054	7.4	7.391	.649	8.129	77.27	973.9	+23.7
30	1.058	7.9	7.919	.698	8.113	83.56	971.8	+23.0
32	1.062	8.5	8.446	.747	8.097	89.49	969.9	+22.3
34	1.066	9.0	8.974	.797	8.081	95.42	968.0	+21.6
36	1.070	9.5	9.502	.847	8.064	101.4	965.8	+20.9
38	1.074	10.0	10.030	.897	8.047	107.5	963.8	+20.2
40	1.078	10.5	10.558	.948	8.030	113.5	961.8	+19.4
42	1.082	11.0	11.086	.999	8.012	119.6	959.6	+18.7
44	1.086	11.5	11.614	1.050	7.994	125.8	957.2	+17.9
46	1.090	12.0	12.142	1.102	7.976	132.0	955.3	+17.1
48	1.094	12.5	12.670	1.154	7.957	138.2	953.0	+16.2
50	1.098	12.9	13.198	1.207	7.937	144.5	950.6	+15.4
52	1.102	13.4	13.725	1.260	7.918	150.9	948.5	+14.5
54	1.106	13.9	14.253	1.313	7.898	157.2	946.2	+13.7
56	1.110	14.4	14.781	1.366	7.878	163.6	943.7	+12.8
58	1.114	14.8	15.309	1.420	7.858	170.1	941.3	+11.8
60	1.118	15.3	15.837	1.475	7.836	176.7	938.8	+10.9
62	1.122	15.8	16.365	1.529	7.815	183.2	936.2	+ 9.9
64	1.126	16.2	16.893	1.584	7.794	189.8	933.7	+8.9
66	1.130	16.7	17.421	1.639	7.772	196.5	931.2	+7.9
68	1.135	17.2	17.949	1.697	7.755	203.2	928.9	+6.8
70	1.139	17.7	18.477	1.753	7.733	209.9	926.3	+5.7
72	1.143	18.1	19.004	1.809	7.710	216.7	923.7	+4.6
74	1.147	18.6	19.532	1.866	7.686	223.5	921.0	+3.4
76	1.152	19.1	20.060	1.925	7.669	230.6	918.6	+2.2
78	1.156	19.6	20.588	1.982	7.645	237.4	915.9	+1.0
80	1.160	20.0	21.116	2.040	7.620	244.4	912.9	— .4
82	1.164	20.4	21.644	2.098	7.596	251.5	910.5	—1.6
84	1.169	21.0	22.172	2.158	7.577	258.5	907.7	—3.0
86	1.173	21.4	22.700	2.218	7.551	265.7	904.6	—4.4
88	1.178	21.9	23.228	2.279	7.531	272.9	902.1	—5.8
**88.3	1.179	22.0	23.310	2.288	7.528	274.1	901.6	—6.0**
90	1.182	22.3	23.755	2.338	7.506	280.1	899.1	—1.1
92	1.186	22.7	24.283	2.398	7.479	287.4	896.0	+4.8
94	1.191	23.3	24.811	2.459	7.460	294.7	893.5	+11.1
95	1.193	23.5	25.075	2.491	7.444	298.4	891.7	+14.4
96	1.195	23.7	25.339	2.522	7.430	302.1	890.1	+18.0
97	1.197	23.9	25.603	2.552	7.417	305.8	888.6	+21.6
98	1.200	24.2	25.867	2.585	7.409	309.6	887.4	+25.5
99	1.202	24.4	26.131	2.616	7.394	313.4	885.7	+29.8
99.6	1.203	24.5	26.285	2.634	7.386	315.4	884.8	+32.3†
100	1.204	24.6	26.395	2.647	7.380	317.1	884.2	+60.0‡

The above table applies to brine tested at the temperature of 60° F.
For brine tested at a warmer or colder temperature than 60° F see Table of Salometer Corrections on reverse side of this card.
*Temperature at which freezing begins. Ice forms, brine concentrates, and the freezing point lowers to eutectic.
**Eutectic point. For brines stronger than eutectic, the temperatures shown are the saturation temperatures for sodium chloride dihydrate.
Brines stronger than eutectic deposit excess sodium chloride as dihydrate when cooled, and freeze at eutectic.
†Transition temperature from anhydrous salt to dihydrate.
‡Saturated brine at 60° F.

MORTON SALT COMPANY • Industrial Products Division
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CHICAGO, ILLINOIS 60606

SALOMETER CORRECTION CHART

When brines are tested at temperatures other than 60° F, the recorded corrections in the chart below should be applied to readings before entering the salometer table on the reverse side of card.

BRINE READING IN DEGREES SALOMETER	SODIUM CHLORIDE SOLUTIONS				
	TEMPERATURE AT WHICH BRINE IS TESTED: °F.				
	0° F.	20° F.	40° F.	80° F.	100° F.
100°	−6.6	−4.9	−2.7	+3.0	+6.0
80°	−6.3	−4.6	−2.5	+2.7	+5.6
60°		−4.0	−2.2	+2.4	+5.0
40°		−2.7	−1.7	+2.1	+4.4
20°		−1.5	−1.0	+1.5	+3.3
0°			−0.7	+0.7	+1.8

In cool brines subtract the correction from reading.
In warm brines add the correction to reading.

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Morton's Purex Salt and Morton Culinox '999' Salt are produced in most of Morton's plants. The Morton Brinemaker Model EP has been designed especially to produce brine from Purex and Culinox Salt. The 100% solubility of these evaporated salts permits continual brine production with no shutdown necessary for cleaning purposes.

Rock salt, of course, varies in purity from one salt producing region to another. If rock salt is used for brine-making for which The Morton Brinemaker Model RP has been specifically designed, cleaning of the Brinemaker will be necessary for continued proper operation.

STRATEGIC LOCATION OF MORTON PLANTS

The strategic locations of the Morton Salt Company's ten plants assure all brine users, regardless of where they are located, of the speediest and most economical service possible in the delivery of their salt supply.

- NEWARK, CALIFORNIA
- SALTAIR, UTAH
- HUTCHINSON, KANSAS
- GRAND SALINE, TEXAS
- WEEKS ISLAND, LOUISIANA
- MANISTEE, MICHIGAN
- PORT HURON, MICHIGAN
- RITTMAN, OHIO
- SILVER SPRINGS, NEW YORK
- FAIRPORT, OHIO

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 05 **Name** BACTERIOLOGICAL ANALYSIS OF SEAFOOD PRODUCTS

Date March, 1980 **Issue** Draft **Page** 1 **of** 13

I. PREPARATION OF MEDIAS (AOAC, 13th Edition, 1980, 46.013)

A. Sample Dilution Solution

1. Butterfield's Phosphate Buffer Solution.

a. Stock Solution:

Dissolve 34.0 g KH_2PO_4 in 500 ml distilled water and dilute to 1 liter. (Adjust pH to 7.2 with 1 M NaOH). Store in refrigerator.

b. Dilutent.

Dilute 2.5 ml stock solution to 2 liters water.

Dispense into:

(1) 99 ml dilution bottles to the line.

(2) Blender jars to 225 ml volume.

Autoclave for 15 minutes at 121°C.

2. Lactose Broth.

Dissolve 26 g Lactose Broth medium in 2 liters of distilled water.

Dispense into:

(1) 99 ml dilution bottles to the line.

(2) Blender jars to 225 ml volume.

Autoclave for 15 minutes at 121°C.

B. Total Plate Count Media

1. Standard Methods Agar.

Suspend 47.0 g Standard Methods Agar in 2 liters of distilled water. Heat with frequent agitation and boil for one minute. Dispense and sterilize by autoclaving at 121°C for 15 minutes. Allow approximately 17 ml Standard Methods Agar (Tryptane Glucose Yeast Agar) per plate. Pouring temperature = 45°C ° 1%.

C. Coliform Medias and Reagents

1. Lauryl Sulfate Tryptose Broth.

Dissolve 71.2 g Lauryl Sulfate Tryptose medium in 2 liters distilled water. Dispense 10 ml portions into 16 x 150 test tubes with small 6 x 50 inverted tubes inside. Sterilize by autoclaving 15 minutes at 121°C. Store in cool, dark place.

2. E. Coli Broth

Dissolve 37.0 g EC medium in 1 liter distilled water. Dispense 10 ml portions into 16 x 150 tubes with 6 x 50 inverted tubes inside. Autoclave 15 minutes at 121°C. Store in cool, dark place.

3. Brilliant Green Lactose Bile (BGLB) Broth.

Dissolve 40 g of BGLB medium in 1 liter distilled water. Dispense 10 ml portions into 16 x 150 tubes with 6 x 50

inverted tubes inside. Autoclave 15 minutes at 121°C. Store in cool, dark place.

4. Levine's Eosin Methylene Blue Agar

Suspend 27.5 g medium in 1 liter distilled water, heat with frequent agitation, and boil for 1 minute. Dispense and sterilize at 121°C for 15 minutes. Cool the sterile fluid agar to about 45°C and agitate gently before pouring plates. Store poured plates in refrigerator.

D. Staphylococci Aureas Media

1. Baird-Parker Medium (Egg tellurite glycine pyruvate agar ETGPA).

Dilute 12.6 g medium to 2 liter with distilled water, heat with frequent agitation, and boil for 1 minute. Autoclave for 15 minutes at 121°C. Cool to 50 - 55°C in a waterbath and aseptically add 100 ml warmed Bacto EY Tellurite enrichment. Mix well, avoiding bubbles, and pour into sterile petri dishes. Store poured plates in refrigerator.

2. Coagulase Plasma

Add 15 ml sterilized distilled water to coagulase plasma with EDTA, and shake until dissolved. Unused plasma may be refrigerated for 2 days or frozen.

3. Brain Heart Infusion Broth

Dissolve 37 g of medium in 1 liter distilled water. Autoclave 15 minutes at 121°C.

4. Trypticase Soy Broth - 10% NaCl.

Add 95 g NaCl and 30 g Trypticase Soy Broth to 1 liter distilled water. Dispense into 16 x 125 mm test tubes to a depth of 5 - 8 cm. Autoclave 15 minutes at 121°C.

II. PREPARATION OF SAMPLE (AOAC, 13th Edition, 1980, 46.014)

A. Aseptically weigh 25 g frozen sample into a sterile blender jar containing 225 ml sterile phosphate buffer solution or lactose broth. Blend 2 minutes. (If necessary, frozen sample may be tempered by holding $\geq$ 18 hours at 2-5°C). Not more than 15 minutes should elapse from time sample is blended until all dilutions are in appropriate media.

B. After sample has been blended yielding a 10^{-1} (1:10) dilution, pipet 11 ml into 99 ml sterile phosphate buffer solution or lactose broth to make a 10^{-2} (1:100) dilution. Shake the bottle 25 times. (For most fresh frozen products this is the highest dilution required unless contamination is suspected). To make a 10^{-3} (1:1000) dilution pipet 11 ml from 10^{-2} dilution into 99 ml buffer solution.

III. AEROBIC PLATE COUNT (AOAC, 13th Edition, 1980, 46.015)

A. Media.

1. Standard Methods Agar.

B. Procedure

Seed duplicate petri plates in dilutions of 10^{-1} (1:10), 10^{-2} (1:100) and 10^{-3} (1:1000), using Standard Methods Agar. Pipet 1 ml appropriate dilution into each plate and add molten agar (cooled to 42-45°C). (0.1 ml of 10^{-2} dilution will yield a 10^{-3} dilution). Incubate 48 ± 2 hour at 35°C and count duplicate plates in suitable range (30 - 300 colonies). If plates do not contain 30 - 300 colonies, record dilution counted and note number of colonies found. Average counts obtained and report as aerobic plate count per gram.

C. Tolerance

APC = 50,000/gram = 5.0×10^4/gram.

IV. COLIFORM GROUP AND E. COLI (AOAC, 13th Edition, 1980, 46.016)

A. Media

1. LST Broth.
2. EC Broth.
3. BGLB Broth.
4. Levine's EMB Agar.

B. Procedure

1. Seed 3 tube most probable number (MPN) series into lauryl sulfate tryptose broth, using 1 ml inocula of 1:10, 1:100 and 1:1000 dilutions, using triplicate tubes at each dilution. (0.1 ml of 1:100 dilution will yield a 1:1000 dilution).
2. Incubate 24 hours at 35°C. Examine for gas formation as evidenced by displacement of liquid in inverted tube or by vigorous effervescence when tubes are shaken gently. Transfer 3 mm loopfuls from gassing tubes to BGLB and EC broth at time gas formation is noted. Reexamine LST tubes at 48 hours for gas formers not detected at 24 hours. Using MPN Table compute most probable number coliforms on basis on number of LST tubes producting gas by end of incubation period. Report gas positive results as MPN presumptive coliforms per gram.
3. Incubate BGLB broth 48 ± 2 hours at 35°C. Using MPN Table compute MPN on basis of number of BGLB tubes producing gas by end of incubation period. Report as MPN coliforms per gram.
4. Incubate EC Broth 24 hours at 45.5 ± 0.05°C (or 44.7 ± 0.2°C for Canadian standards) in covered water bath. Sumberge broth tubes in bath so that water level is above highest level of medium. Examine tubes for gas formation at 24 or 48 hours. Record gas formers and report gas positive results as MPN presumptive fecal coliforms per gram.

5. Streak gas-positive tubes on EMB agar plates, and incubate 24 ± 2 hours at 35°C. Record discrete colonies with or without sheen, showing nucleated centers, as MPN typical E. coli per gram.

6. The above tests are sufficient to demonstrate fecal coliform contamination. If further identification of E. Coli is required, refer to AOAC, 13th Edition, 1980, 46.016.

C. Tolerance

MPN E. Coli = 3.6/gram.

D. See attached chart for Coliform Group and E. Coli procedure.

V. STAPHYLOCOCCUS AUREUS (AOAC, 13th Edition, 1980, 46.075).

A. Media:

1. Baird-Parker Medium.
2. Coagulase Plasma.
3. Brain Heart Infusion-Broth.
4. Trypticase Soy Broth - 10% NaCl.

B. Procedure:

1. For the 1:10 and 1:100 dilutions (usually 1:10 dilution is sufficient) pipet 1 ml sample dilution to 3 plates of Baird-Parker medium, distributing the 1 ml equitably among the 3 plates (e.g., 0.4 ml - 0.3 ml - 0.3 ml).
2. Spread inoculum over surface of agar using sterile, bent glass streaking rods. Avoid extreme edges of plate. Retain plates in upright position until inoculum is absorbed by medium. Invert plates and incubate at 35-37°C for 45-48 hours.
3. Count only colonies which appear to be Staphylococcus Aureus. Colonies of Staphylococcus aureus are circular, smooth, convex, moist, 2-3 mm in diameter, black with light-colored margin surrounded by opaque zone and frequently with outer clear zone; Colonies have buttery to gummy consistency when touched with innoculating needle. Add number of staphylococcus appearing colonies on triplicate plates and multiply by sample dilution dilution factor and report as staph APC/gram.

4. From each plate showing growth select ≥ 1 staph appearing colony and test for coagulase production.

5. Inoculate each colony type into separate 10 x 75 mm sterilized tube containing 0.2 ml sterile brain heart infusion broth and emulsify thoroughly. Incubate 18-24 hours at 35-37°C. Add 0.5 ml sterile reconstituted coagulase plasma and mix thoroughly. Incubate at 35-37°C and examine periodically over 6 hour interval for clot formation. Any degree of clot formation is considered a positive test for Staphylococcus aureus.

6. Add number of colonies on triplicate plates represented by colonies giving positive coagulase test and multiply by sample dilution factor. Report this number as coagulase (+) Staphylococcus aureus/gram.

7. The above test for Staphylococcus aureus is adequate in most cases. If a more sensitive test is required refer to procedure 46.017 Coagulase - Positive Staphylococcus aureus using Trypticase Soy Broth in AOAC 13th Edition, 1980.

C. Tolerance

Coagulase Positive Staph - 50/gram.

D. See Chart for Staphylococcus Aureus Procedure, attached.

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 05 **Name** BACTERIOLOGICAL ANALYSIS OF CRAB PRODUCT

Date March, 1980 **Issue** Draft **Page** 10 **of** 13

STAPHYLOCOCCUS AUREUS PROCEDURE

Distribute 1 ml of 10^{-1} dilution among 3 Baird Parker Medium Plates. Incubate 48 hr at 35°C

- No. Staph appearing Colonies
- Count number of staph appearing colonies and compute number of Staph APC/g
  - Inoculate staph appearing colonies into separate tubes of 0.2 ml brain heart infusion broth. Incubate 24 hr at 35°C.
    - Add 0.5 ml coagulase plasma. Mix thoroughly. Incubate 6 additional hours at 35°C and check for coagulation. Compute number of coagulase (+) S. aureus/g.

CASTLE & COOKE FOODS

TESTING PROCEDURE

VI. COLIFORM GROUP AND E. COLI IN WATER SAMPLES (STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWATER, 14th Edition, 1975).

A. Media

1. LST Broth.
2. EC Broth.
3. BGLB Broth.
4. Levine's EMB Agar.

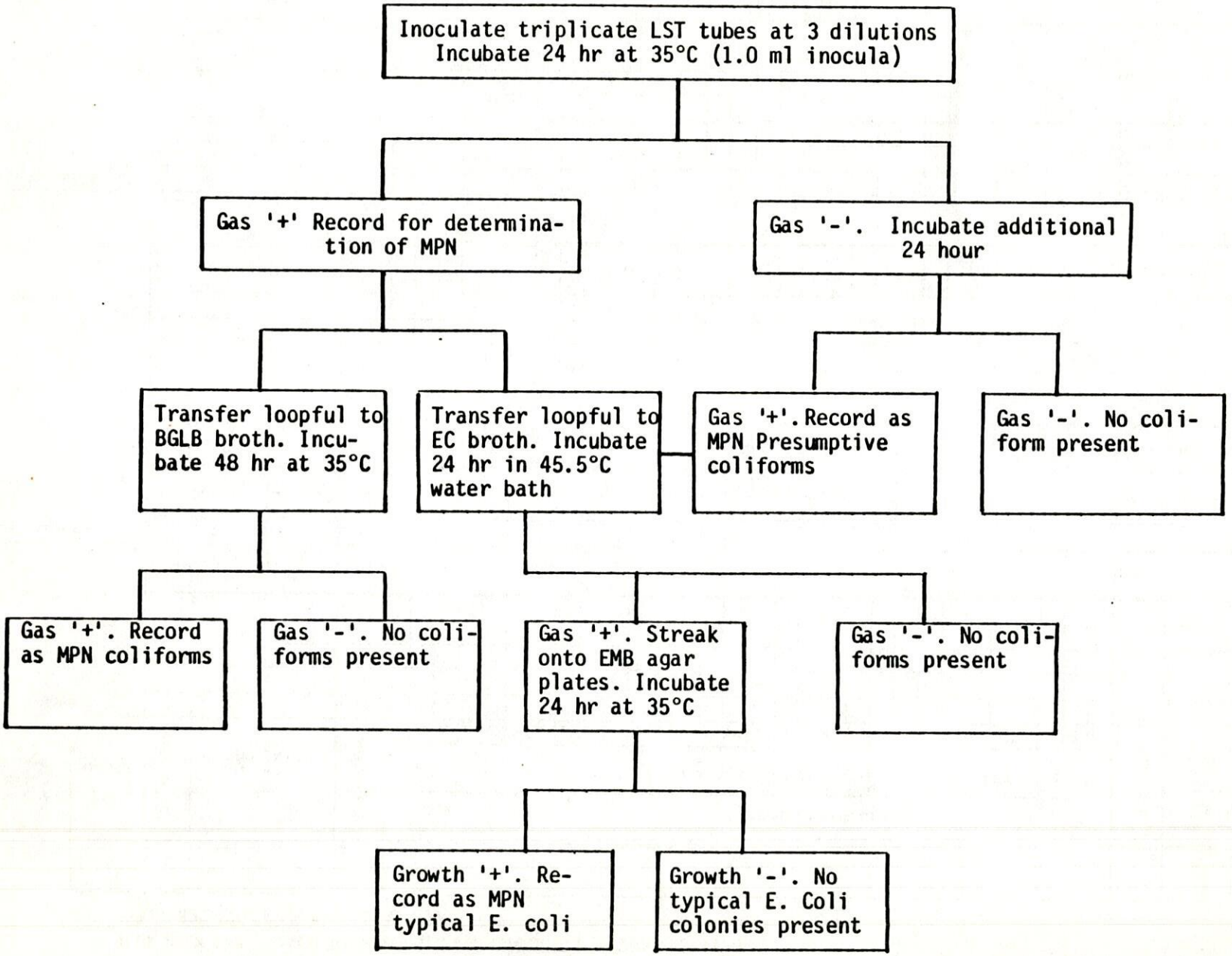
B. Procedure:

1. Seed 5 tube most probable number (MPN) series into lauryl sulfate tryptose broth using 1 ml inocula of 1:0 (undiluted water sample) 1:10, and 1:100 dilutions using 5 tubes at each dilution. (0.1 ml of 1:10 dilution will yield a 1:100 dilution).
2. Proceed as in Procedure IV. Coliform Group and E. Coli, except use 5 tube MPN Tables to calculate MPN coliforms per 100 ml sample.

VII. MOST PROBABLE NUMBER (MPN) CHARTS:

See Attached.

COLIFORM GROUP AND E. COLI PROCEDURE


Inoculate triplicate LST tubes at 3 dilutions
Incubate 24 hr at 35°C (1.0 ml inocula)
Gas '+' Record for determination of MPN
Gas '-'. Incubate additional 24 hour
Transfer loopful to BGLB broth. Incubate 48 hr at 35°C
Transfer loopful to EC broth. Incubate 24 hr in 45.5°C water bath
Gas '+'. Record as MPN Presumptive coliforms
Gas '-'. No coliform present
Gas '+'. Record as MPN coliforms
Gas '-'. No coliforms present
Gas '+'. Streak onto EMB agar plates. Incubate 24 hr at 35°C
Gas '-'. No coliforms present
Growth '+'. Record as MPN typical E. coli
Growth '-'. No typical E. Coli colonies present

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DATE

ANALYST (S)

SAMPLE NUMBER	SUB NO.	DILUTION	APC COLONIES PER PLATE	DILUTION	COLIFORM GROUP LST A	B	C	COLIFORM GROUP BG A	B	C	ESCHERICHIA COLI EC A	B	C	EMB A	B	C	I M V C A	I M V C B	I M V C C	LST A	B	C	GRAM STAIN A	B	C	DILUTION	STAPH APC COLONIES PER PLATE	COAGULASE POSITIVE APC COLONIES PER PLATE
	APC/g:			MPN/g:							MPN/g:															APC/g:		
	APC/g:			MPN/g:							MPN/g:															APC/g:		
	APC/g:			MPN/g:							MPN/g:															APC/g:		
	APC/g:			MPN/g:							MPN/g:															APC/g:		
	APC/g:			MPN/g:							MPN/g:															APC/g:		

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 05 Name BACTERIOLOGICAL ANALYSIS OF SEAFOOD PRODUCT

Date March, 1980 Issue Draft

Most Probable Numbers (MPN) per gram of Sample, Planting 0.1 and 0.001 ml Portions. Three tube method

No. of Positive Tubes			MPN Per Gram	No. of Positive Tubes			MPN Per Gram
0.1 gram	0.01 gram	0.001 gram		0.1 gram	0.01 gram	0.001 gram	
0	0	0	<3	2	0	0	9.1
0	0	1	3.0	2	0	1	14.0
0	0	2	6.0	2	0	2	20.0
0	1	0	3.0	2	1	0	15.0
0	1	1	6.1	2	1	1	20.0
0	1	2	9.2	2	1	2	27.0
0	1	2	12.0	2	1	3	34.0
0	2	0	6.2	2	2	0	21.0
0	2	1	9.3	2	2	1	28.0
0	2	2	12.0	2	2	2	35.0
0	2	3	16.0	2	2	3	42.0
0	3	0	9.4	2	3	0	29.0
0	3	1	13.0	2	2	1	36.0
0	3	2	16.0	2	3	2	44.0
0	3	3	19.0	2	3	3	53.0
1	0	0	3.6	3	0	0	23.0
1	0	2	7.2	3	0	1	39.0
1	0	2	11.0	3	0	2	64.0
1	0	3	15.0	3	0	3	95.0
1	1	0	7.3	3	1	0	43.0
1	1	1	11.0	3	1	1	75.0
1	1	2	15.0	3	1	2	120.0
1	1	3	19.0	3	1	3	160.0
1	2	0	11.0	3	2	0	93.0
1	2	1	15.0	3	2	1	150.0
1	2	2	20.0	3	2	2	210.0
1	2	3	24.0	3	2	3	290.0
1	3	0	16.0	3	3	0	240.0
1	3	1	20.0	3	3	1	460.0
1	3	2	24.0	3	3	2	1,100.0
1	3	3	29.0	3	3	3	>1,100.0

ITTEN BY:	TECHNICAL DIRECTOR:	QUALITY CONTROL DIRECTOR:	V.P. TECHNICAL SERVICES:

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 05 Name BACTERIOLOGICAL ANALYSIS OF SEAFOOD PRODUCTS

Date March, 1980 Issue Draft Page 13 of 13

Most Probable Number (MPN) per 100 ml of Sample,
Planting 1.0, 0.1, and 0.01 ml Portions. Five tube Method

Positive No. of Tubes			MPN per 100 ml	Positive No. of Tubes			MPN per 100 ml
5 of 1 ml Each	5 of 0.1 ml Each	5 of 0.01 ml Each		5 of 1 ml Each	5 of 0.1 ml Each	5 of 0.01 ml Each	
0	0	0	<20				
0	0	1	20	4	2	1	260
0	1	0	20	4	3	0	270
0	2	0	40	4	3	1	330
				4	4	0	340
1	0	0	20				
1	0	1	40	5	0	0	230
1	1	0	40	5	0	1	310
1	1	1	60	5	0	2	430
1	2	0	60	5	1	0	33[illegible]
				5	1	1	460
2	0	0	50	5	1	2	630
2	0	1	70				
2	1	0	70	5	3	0	490
2	0	0	50	5	1	2	630
2	0	1	70				
2	1	0	70	5	2	0	490
2	1	1	90	5	2	1	700
2	2	0	90	5	2	2	940
2	3	0	120	5	3	0	790
				5	3	1	1100
3	0	0	80	5	3	2	1400
3	0	1	110	5	3	3	1800
3	1	0	110	5	4	0	1300
3	1	1	140	5	4	1	1700
3	2	0	140	5	4	2	2200
3	2	1	170	5	4	3	2800
3	3	0	170	5	4	4	3500
4	0	0	130	5	5	0	2400
4	0	1	170	5	5	1	3500
4	1	0	170	5	5	2	5400
4	1	1	210	5	5	3	9200
4	1	2	260	5	5	4	16000
4	2	0	220	5	5	5	≥24000

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CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 06 Name DRAINED WEIGHT OF CRAB MEAT PRODUCTS

Date March, 1980 Issue Draft Page 1 of 3

I. Materials:

A. Metal corrosion resistant, with #8 screen.

B. Timer with second hand.

C. Balance type scale accurate to 0.1 ounce.

II. Procedure:

A. Frozen Drain Weight and Block Evaluation Procedures.

1. Strip frozen block of pan and liner.
2. Note appearance of block and make necessary comments on Drained Weight form. Count number of merus segments and note on form.
3. Cut block into three equal portions (markings on liner represent five-pound segregations).
4. Weigh each five-pound block, free of liner, and record.
5. Place each block in non-permeable plastic bag and let thaw for approximately 24 hours.
6. After thawing, place block on a #8 screen and let drain for exactly two minutes.
7. Weigh block in a pan or tray. This is the drained weight.
8. Separate merus, white, and red meat and weigh. Record this data on form.
9. Carefully look through meat and take out and record numbers and types of defects. (Defects are anything which is not wholesome crab meat).

10. Be sure to mark percentages of drained weight for each type of meat and the total.

B. Unfrozen Drain Weight Procedures.

1. Weigh pan and liner and meat and record on Drained Weight Form.
2. Place #8 long john screen over pan.
3. Invert at 15° - 25° angle and let drain for exactly two minutes.
4. Weigh pan with liner and meat and record as gross drained weight.
5. Separate merus, white, and red meat and weigh each. Record this on form.
6. Weigh pan and liner and record both.
7. Subtract the weight of pan and liner. This is the drained weight.
8. Be sure to mark percentages of drained weight for each type of meat and total.

C. Defects.

1. Defect - any barnacle, tendon, shell, gill, or other extraneous material. Defect less than 1 cm in any dimension are defined as minor; defects larger than 1 cm

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 06 Name DRAINED WEIGHT OF CRAB MEAT PRODUCTS

Date March, 1980 Issue Draft Page 3 of 3

are defined as major. Three minor defects are equivalent to one major defect.

2. Defect limits are:

   a. One per pound for king crab meat.

   b. Five per pound for snow crab meat.

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TESTING PROCEDURE

T. P. No. 07 Name SALT DETERMINATION (SILVER NITRATE TITRATION METHOD)

Date March, 1980 Issue Draft

I. SILVER NITRATE TITRATION METHOD

A. Materials

1. 50 ml buret.
2. 0.10N NaCl (TP NO. 8)
3. 0.10N $AgNO_3$ (TP NO. 8).
4. One pint blender jars.
5. 100 ml beakers.
6. Distilled water.
7. $K_2Cr_2O_7$ indicator (potassium dichromate). (TP NO. 8).

B. Procedure.

1. Standardization of $AgNO_3$.

a. Rinse buret with distilled water and let drain.

b. Measure 10 ml 0.1N NaCl into beaker, dilute to 100 ml with distilled water.

c. Add 6 drops $K_2Cr_2O_7$ indicator.

d. Add $AgNO_3$ to buret.

NOTE: $AgNO_3$ breaks down rapidly in light. Keep in tightly closed brown bottles. Use as soon as possible after dispensing into buret.

e. Titrate to orange-brown end point.

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2. Salt Determination (Titration Method).

   a. Thaw sample.

   b. Weigh out 25 grams sample into blender jar.

   c. Add 225 ml distilled water.

   d. Blend 2 minutes.

   e. Divide blended sample into 50 ml portions in glass beakers.

   f. Dilute each 50 ml portion to 100 ml with distilled water.

   g. Add 6 drops $K_2Cr_2O_7$ indicator.

   h. Titrate to orange-brown end point with standardized $AgNO_3$.

   i. Titrate duplicate or triplicate samples as needed to get consistent results.

3. Calculations.

   a. Standardization of $AgNO_3$.

$$N\ AgNO_3 = \frac{(10\ ml.\ NaCl)\quad (0.1\ N\ NaCl)}{\_\_\_\_\ ml.\ AgNO_3}$$

   b. Salt Determination.

$$\%\ NaCl\ in\ sample = \frac{(\_\_\_\ ml.\ AgNo_3)\ (\_\_\_\ N\ AgNO_3)\ (58.45)(100)}{1000\ (weight\ of\ sample)}$$

II. CHLORIDE PROBE METHOD

A. Introduction

This method determines the concentration of free chloride ions in aqueous solutions using a chloride specific electrode coupled with a digital pH/MV meter. In theory, the chloride ions in solution react with silver ions in the electrode membrane, generating an ionic potential across the electrode membrane. The ionic potential is recorded as a milivolt reading by the pH/MV meter.

A calibration curve is constructed by plotting milivolt readings of standard chloride solutions against their concentrations on semilogarithmic paper. For each sample a milivolt reading is obtained, and corresponding chloride concentration is read directly off the calibration curve.

Both standard solutions and samples are prepared by diluting 1:10 with distilled water. Ionic strength adjustor (ISA) is added to all standards and samples to maintain a constant background ionic strength.

SALT DETERMINATION REPORT

CHLORIDE PROBE METHOD

PLANT:_____ ANALYST:_____ DATE: _____

0.1% Standard = _____ MV
1.0% Standard = 0.0 MV
10.0% Standard = _____ MV

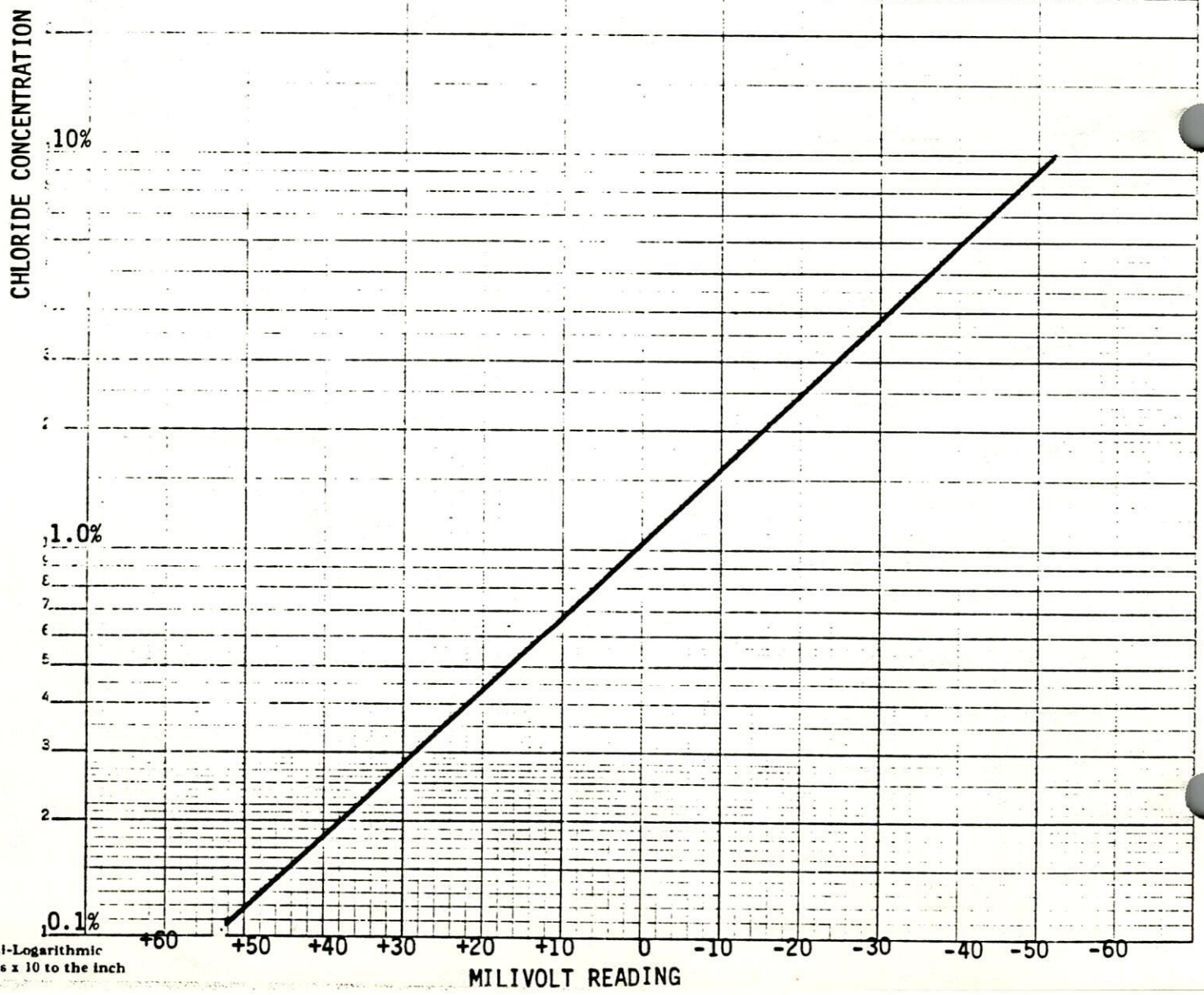
SAMPLE	DAY CODE	Milivolt Reading	% Chloride	FLAVOR

SALT DETERMINATION

CHLORIDE PROBE METHOD

SAMPLE GRAPH

EXAMPLE: Milivolt reading of -18 corresponds to chloride concentration of 2.2%.



Semi-Logarithmic
4 Cycles x 10 to the inch

B. Materials

1. Model 94-17 Orion chloride electrode.
2. Model 90-02 Orion double - junction reference electrode.
3. Corning digital pH/MV meter.
4. Magnetic stirrer.
5. Beakers.
6. Graph paper - 4 cycle, semilogarithmic.
7. Polishing strips Orion Catalog Number 94-82-01.
8. Blender jars (1/2 pint).
9. Distilled water.
10. Ionic strength adjustor solution (ISA) (TP No. 8).
11. '10%' chloride standard (TP NO. 8)
12. '10%' chloride standard (TP NO. 8)
13. '0.1%' chloride standard (TP NO. 8)
14. Reference elctrode filling solutions.

C. Procedure.

1. Connecting Electrodes to Meter

   a. Remove protective rubber cap covering membrane of chloride electrode.

   b. Fill reference electrode according to Orion operating instructions.

c. Insert electrode connectors into appropriate jacks on digital pH/MV meter.

2. Calibration of Electrodes

a. Make up '10%', '1%' and '0.1%' chloride standards, and ISA solution (TP NO. 08).

b. Put 100 ml '1%' standard and 2 ml ISA into a beaker. Stir with magnetic stirrer. Turn meter function switch to REL MV position. Place electrodes in solution. Press Stand-By switch. Set the reading to 000 MV by turning the calibration control. Press Stand-By switch. Remove electrodes, rinse, dry.

c. Put 100 ml '10%' standard and 2 ml ISA into beaker. Stir with magnetic stirrer and take milivolt reading (approximately -52 milivolts).

d. Put 100 ml '0.1%' standard and 2 ml ISA into beaker. Stir with magnetic stirrer and take milivolt reading (approximately +52 milivolts).

e. Plot standard milivolt readings on X axis against standard concentrations on Y axis to make calibration curve. (See sample graph).

3. Chloride Determination of Samples

a. Weigh 10 g sample into 1/2 pint blender jar. Add 90 ml distilled water and 2 ml ISA. Blend 2 minutes.

b. Stir blended sample with magnetic stirrer and take milivolt reading.

c. Plot milivolt reading on calibration curve and read off corresponding chloride concentration.

4. Measuring Hints

a. For maximum precision allow all standards and samples to come to ambient temperature before measurement.

b. Stir sample and standards during measurement with magnetic stirrer.

c. Rinse the electrodes and blot dry with clean, dry tissue between measurements to prevent solution carryover.

d. Press Stand-By switch before removing electrode from solution.

5. Reference

Orion Ionalyzer Instruction Manual Halide Electrodes.
Orion Research Incorporated, 380 Putman Avenue, Cambridge, Massachusetts 02139 (617) 864-5400.

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 08 Name STANDARD SOLUTIONS

Date April, 1981 Issue Draft Page 1 of 3

I. SALT DETERMINATION

A. $AgNo_3$ (Silver Nitrate).

Dissolve 17.0 grams reagent grade silver nitrate in 1,000 ml distilled water. Store in tightly-capped brown glass bottles in the dark. Standardize with standard NaCl solution prior to use. (TP NO. 7).

B. Potassium Dichromate Indicator Solution

Dissolve 63 g $K_2Cr_2O_7$ in 1,000 ml distilled water. Add a few drops of 0.10 N $AgNO_3$ until a definite red precipitate persists. Let stand to settle, filter, and store in glass dropping bottle.

C. Standard Sodium Chloride

Dry about 15 g NaCl to constant weight. Cool and weigh out 5.84 g. Dissolve in distilled water and dilute to 1,000 ml.

Standardization: Place 10.0 ml standard NaCl solution in a 150 ml erlenmeyer flask. Dilute to 100 ml with distilled water. Add 6 drops of dichromate indicator and titrate with $AgNO_3$ solution until a red precipitate just forms. Stopper flask with rubber stopper and shake vigorously to break cuds of AgCl. Wash down stopper and continue titration to orange brown end point.

$$N\ NaCl = \frac{(N\ AgNO_3)\ \ (ml\ NaCl)}{(ml\ AgNO_3)}$$

WRITTEN BY:	TECHNICAL DIRECTOR:	QUALITY CONTROL DIRECTOR:	V.P. TECHNICAL SERVICES:

D. Ionic Strength Adjustor Solution (ISA).

Prepare 5 M $NaNO_3$ by dissolving 42.5 g sodium nitrate in 100 ml distilled water. 2 ml ISA is added to 100 ml standard or sample solutions to bring the background ionic strength to 0.1M.

E. 10% Chloride Standard

Place 16.5 g NaCl in a 1 liter volumetric flask. Add about 500 ml distilled water, swirl to dissolve, and dilute to mark with distilled water. (This is actually a 10% chloride solution diluted 1:10).

F. 1% Chloride Standard

Pipet 10 ml 10% chloride standard and dilute to 100 ml with distilled water. (This is actually a 1% chloride solution diluted 1:10).

G. 0.1% Chloride Standard

Pipet 10 ml 1% chloride standard and dilute to 100 ml with distilled water. (This is actually a 0.1% chloride solution diluted 1:10).

II. CHEMICAL OXYGEN DEMAND (COD)

A. Standard Potassium Dichromate Solution. 0.250N.

Dissolve 12.259 g $K_2Cr_2O_7$ primary standard grade, previously dried at 103°C for two hours, in distilled water and dilute to 1,000 ml.

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 08 Name STANDARD SOLUTIONS

Date April, 1981 Issue Draft Page 3 of 3

B. Sulfuric Acid Reagent

Concentrated H_2SO_4 containing 22 g silver sulfate, Ag_2SO_4; per 4 kg (9-lb) bottle (1 to 2 days required for dissolution).

C. Standard Ferrous Ammonium Sulfate Titrant, 0.01N:

Dissolve 39 g $Fe(NH_4)_2(SO_4)_2.6H_2O$ in distilled water. Add 20 ml concentrated H_2SO_4, cool, and dilute to 1,000 ml. Standardize this solution daily against the standard $K_2Cr_2O_7$ solution.

Standardization: Dilute 10.0 ml standard $K_2Cr_2O_7$ solution to about 100 ml. Add 30 ml concentrated H_2SO_4 and cool. Titrate with the ferrous ammonium sulfate titrant, using 2 to 3 drops (0.10 to 0.15 ml) ferroin indicator.

D. Ferroin Indicator Solution

Dissolve 1.485 g 1,10 - phenanthroline monohydrate, together with 695 mg $FeSO_4.7H_2O$ in water and dilute to 100 ml. This indicator solution may be purchased already prepared.

III. ORTHOTOLIDINE REAGENT FOR RESIDUAL CHLORINE

Dissolve 1.35 g orthotolidine dihydrochloride in 500 ml distilled water. To this solution, with constant stirring, add a mixture of 350 ml distilled H_2O and 150 ml concentrated HCl. Store in brown bottles in the dark. Store above 0°C. Do not use more than six months.

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 09 Name METHODOLOGY FOR PERCENT MEAT

Date April, 1981 Issue _____ Page 1 of 3

I. METHODOLOGY FOR FROZEN

A. Net Weight of Glazed Product.

1. Equipment

a. Scale.

b. U.S. No. 8 sieve 12" diameter.

c. Cold water spray.

2. Procedure

Remove section from low temperature storage, open immediately, and place minimum 3 pound sample, gills removed, beneath gentle cold water spray. Agitate carefully so product is not broken. Spray until all ice that can be seen or felt is removed. Transfer product to circular #8 sieve, 12" diameter. Without shifting, incline sieve at angle of 17° to 20° to facilitate draining, drain exactly two (2) minutes with timer. Immediately transfer product to tared pan (B) and weigh (A). Net weight of section = A - B.

Definition of section - one-half of a whole crab.

B. Percent Meat

1. Equipment.

a. Scale.

b. U. S. No. 8 sieve, 12" diameter.

c. Cold water spray.

2. Procedure

Let sample thaw naturally, not in microwave, to a range of 40° to 50°F. Break the shells after determining net weight, extract the meat and coagulase. Wash residual meat off the shell also. Immediately transfer meat, coagulase and residual meat from shell to sieve. Let drain two (2) minutes at 17° to 20° angle. Transfer to tared pan (D) and weigh (C).

Meat weight = C - D.

Percent meat = $\frac{(C - D)}{(A - B)} \times 100$ or $\frac{\text{meat weight}}{\text{net weight section}}$

II. METHODOLOGY FOR UNFROZEN, NORTHERN OPERATIONS

A. Net Weight of Unfrozen Product

1. Equipment

a. Scale.

b. U.S. No. 8 sieve, 12" diameter.

2. Procedure

Remove sample from ambient tank, after cook. Sample should be natural proportions (example: right half with large claw and arm, and left half with small claw and arm). Remove gills. Transfer to sieve. Let drain for two (2) minutes at 17° to 20° angle. Transfer inshell product to tared pan (B) and weigh (A). Net weight of sample = A - B.

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 09 Name METHODOLOGY FOR PERCENT MEAT

Date April, 1981 Issue _____ Page 3 of 3

B. Percent Meat Procedure

Break the shells. Extract meat and coagulase. Wash residual meat off the shell also. Transfer meat, coagulase and residual meat off shell to No. 8 sieve. Let drain for two (2) minutes at 17° to 20° angle. Transfer to tared pan (D) and weigh (C).

Meat weight = C - D.

$$\text{Percent meat} = \frac{(C-D)\ 100}{(A-B)} \quad \text{or} \quad \frac{\text{meat weight} \times 100}{\text{net weight}}$$

Standards - Alaska King Crab Marketing and Quality Control Board Procedures:

1. The legs shall contain a minimum of 50% meat.
2. The large claws with claw arms shall contain a minimum of 40% meat.
3. The small claws and claw arms shall contain a minimum of 30% meat.

RITTEN BY:	TECHNICAL DIRECTOR:	QUALITY CONTROL DIRECTOR:	V.P. TECHNICAL SERVICES:

2. Composite Samples

a. Take grab samples from effluent sump or outfall using a stainless steel bucket on chain.

b. Take grab samples at 2 hour intervals during processing and clean-up time.

c. As each grab sample is taken, stir with a glass rod and then measure two equal portions and disperse into different composite sample bottles (the size of each portion will depend on the number of samples composited throughout the day to make a total of 1,000 ml, each bottle):

Bottle 1: For BODt, BODs, TSS and pH analyses.

Bottle 2: For TKN and O&G analyses (0.8 ml H_2SO_4 is added to bottle initially to preserve sample).

d. Identify composite samples with plant day code, sampling location and sampling procedure.

e. Store composite sample bottles at $\leq$ 4°C in ice in styrofoam container from time of first sampling until time of analysis (26 ± 2 hr.).

CASTLE & COOKE FOODS

TESTING PROCEDURE

COLLECTION AND PRESERVATION OF WASTE WATER EFFLUENT SAMPLES[1]

T. P. No. 10 Name

Date April, 1981 Issue Draft Page 1 of 2

I. MATERIALS

1. Stainless steel bucket on chain.
2. Glass stirring rod.
3. One (1) liter plastic bottles.
4. Concentrated sulfuric acid H_2SO_4.
5. Styrofoam container and gel ice.
6. Graduated cylinder (250 ml).

II. PROCEDURE

1. Grab Samples

   a. Take grab samples from effluent sump or outfall using a stainless steel bucket on chain.

   b. As each grab sample is taken, stir with a glass rod and then divide into different 1 liter plastic samples bottles.

      Bottle 1: For BODt, BODs, TSS and pH analyses.

      Bottle 2: For TKN and O&G analyses (0.8 ml H_2SO_4 is added to bottle initially to preserve sample).

   c. Identify samples with plant day code, sampling location and sampling procedure.

   d. Store sample bottles at $\leq$ 4°C in ice in styrofoam container from time of sampling until time of analyses. (26 ± 2 hr.).

[1]Adapted from: *Standard Methods for the Examination of Water and Wastewater*, 14th Edition

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TESTING PROCEDURE

T. P. No. 11 Name BIOCHEMICAL OXYGEN DEMAND[1]

Date April, 1981 Issue Draft Page 1 of 7

I. INTRODUCTION

1. The biochemical oxygen demand (BOD) test is an empirical bioassy procedure which measures the dissolved oxygen consumed by microbial life while assimilating and oxidizing the organic matter present. Application of the test to organic waste discharges allows calculation of discharge effects on the receiving water oxygen resources. This procedure determines BOD by measuring dissolved oxygen depletion in the sample over a 5 day period.

2. Two types of biochemical oxygen demand tests are run: BOD total (BODt) and BOD soluble (BODs). BOD total is a measurement of the biochemical oxygen demand of the total effluent. BOD soluble is a measurement of the biochemical oxygen demand of the effluent that has been filtered to remove all suspended solids.

3. The samples are seeded by the addition of settled sewage water that contains bacteria. The purpose of seeding is to introduce into the sample a biological population capable of oxidizing the organic matter in the waste water. The oxygen depletion of the seed is determined and this seed correction factor is subtracted from the oxygen depletion in the samples.

[1]Adapted from: *Standard Methods for the Examination of Water and Wastewater*, 14th Edition, and *Seattle Metro Water Quality Division Procedures Manual*

WRITTEN BY:	TECHNICAL DIRECTOR:	QUALITY CONTROL DIRECTOR:	V.P. TECHNICAL SERVICES:

II. MATERIALS

1. BOD bottles (300 ml) with ground glass stoppers (16 per sample for BODt, 16 per sample for BODs, plus 2 for blanks and 6 for seed dilution series).
2. Incubator (20°C ± 1°C) or refrigerator converted to incubator with a Hack Incutrol/2 temperature regulator.
3. Phosphate buffer solution (pH 7.2).
4. Magnesium sulfate solution.
5. Calcium chloride solution.
6. Ferric chloride solution.
7. pH meter and pH electrodes.
8. Sulfuric acid solution (1N).
9. Sodium hydroxide solution (1N).
10. Distilled water.
11. Carboy (20 liters).
12. Compressed air source.
13. Pipets (10 ml).
14. Sewage seed water.
15. Vacuum filtration assembly for BODs test (the same assembly is used for TSS test):
    a. Glass fiber filter disks, Whatman GF/A 2.1 cm.
    b. Gooch crucibles, 22 mm bottom diameter (3 per sample).

c. Vacuum Flask, 1,000 ml.

d. Gooch crucible adapter, Walter.

e. Vacuum pump.

f. Vacuum tubing.

III. PREPARATION OF DILUTION WATER

1. Wash carboy well and rinse with distilled water.
2. Fill carboy with distilled water (approximately 6 liters per sample if running BODt only, 12 liters per sample if running both BODt and BODs).
3. To carboy add 1 ml each of phosphate buffer solution, magnesium sulfate solution, ferric chloride solution, and calcium chloride solution for each liter in carboy.
4. Aerate dilution water with clean compressed air, or by shaking the partially filled bottle.

IV. PROCEDURE

1. Wash all BOD glassware well and rinse with distilled water.
2. Adjust sample pH to 7.0 ± 0.2 with sulfuric acid solution or sodium hydroxide solution.
3. Dilution water blanks: Fill two marked BOD bottles with dilution water to capacity, stopper, and make air tight water seal on each bottle.
4. Seed dilution series: Make up two sets of the following seed dilution series by pipeting the settled seed into marked BOD bottles:

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 11 Name BIOCHEMICAL OXYGEN DEMAND[1]

Date April, 1981 Issue Draft Page 4 of 7

.3% seed = .9 ml seed

.5% seed = 1.5 ml seed

.7% seed = 2.1 ml seed

Fill bottles with dilution water to capacity, stopper, and make air tight water seal on each bottle.

5. After making up dilution water blanks and seed dilution series add 1 ml seed per liter dilution water in carboy. Use this seeded water for making up sample dilutions.

6. Determine approximate range of sample BOD from historical data or chemical oxygen demand (COD) test results (see Chemical Oxygen Demand Testing Procedure No.). If COD test is used, divide COD value by 2 to estimate BOD. Select the appropriate ranges from the following list so that at least four dilutions can be obtained.

BOD RANGE (mg/1)	SAMPLE AMOUNT (ml)	DILUTION RATIO
200 to 600	3.0	None
2,000 to 6,000	3.0	1:10
20,000 to 60,000	3.0	1:100
120 to 360	5.0	None
1,200 to 3,600	5.0	1:10
12,000 to 36,000	5.0	1:100
60 to 180	10.0	None
600 to 1,800	10.0	1:10
6,000 to 18,000	10.0	1:100
60,000 to 180,000	10.0	1:1,000

40 to 120	15.0	None
400 to 1,200	15.0	1:10
4,000 to 12,000	15.0	1:100
40,000 to 120,000	15.0	1:1,000
30 to 90	20.0	None
300 to 900	20.0	1:10
3,000 to 9,000	20.0	1:100
30,000 to 90,000	20.0	1:1,000
240 to 720	25.0	1:10
2,400 to 7,200	25.0	1:100
24,000 to	25.0	1:1,000
200 to 600	30.0	1:10
2,000 to 6,000	30.0	1:100
20,000 to 60,000	30,000	1:1,000

7. Historical BOD data on Pan-Alaska, Monroe effluent suggests the following sample dilution procedure:

   a. For BOD total test: Pipet 3, 5, 10 and 20 ml of sample into marked BOD bottles. Use four (4) bottles for each dilution.

   b. For BOD soluble test: Filter sample with filtration apparatus. Pipet 3,5, 10 and 20 ml of sample filtrate into marked BOD bottles. Use four (4) bottles for each dilution. (If the BODs and TSS tests are run concurrently the filtrate from the TSS test can be used for the BODs test).

   c. Fill sample BOD bottles to capacity with seeded dilution water. Insert stopper and ensure that no air is trapped in the bottle. Make an air tight water seal on each bottle.

8. Set aside one bottle from each sample dilution, one blank, and one seed dilution series. Allow to stand 15 minutes.

9. Store remaining samples, blank and seed dilution series in an incubator at 20° ± 1° C for 5 days ± 4 hours.

10. Perform dissolved oxygen analysis (DO) on sample, blank and seed dilution series from Step 8. (See Dissolved Oxygen Analysis, TP NO. 12 and NO. 13). Record results.

11. After five (5) days ± 4 hours remove samples, blank and seed dilution series from incubator and perform DO analysis. Record results.

V. CALCULATION

1. Determine the quality of dilution water as follows:

BOD (mg/1) = D_1 Blank - D_2 Blank

Where D_1 blank = Initial DO of blank (mg/1)

D_2 blank = DO of blank after 5 days (mg/1).

A BOD of 0.2 mg/1 or less indicates good quality dilution water.

2. From seed dilution series choose one seed dilution that has 40 - 70% oxygen depletion after incubation to calculate seed correction factor as follows:

Seed correction = $(B_1 - B_2)\ f$

Where B_1 = DO of seed dilution before incubation

B_2 = DO of seed dilution after incubation

f = ration of seed in sample to seed in control

$$= \frac{\% \text{ seed in } D_1}{\% \text{ seed in } B_1}$$

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TESTING PROCEDURE

T. P. No. 11 Name BIOCHEMICAL OXYGEN DEMAND[1]

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3. Determine BOD for samples as follows:

$$\text{BOD (mg/1)} = \frac{(D_1 - D_2) - (B_1 - B_2)\, f}{P}$$

Where D_1 = Initial DO of sample (mg/1)

D_2 = Sample DO after 5 days incubation (mg/1)

$(B_1 - B_2)\, f$ = seed correction

P = Decimal Fraction of sample used, i.e., volume sample/ total volume.

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BOD DETERMINATION

OXYGEN ELECTRODE CALIBRATION

PLANT: _____ ANALYST: _____ DATE: _____

SAMPLE NO: _____ TEST: _____

(5 Day DO Test)

Titrations - - #1 _____ _____

#2 _____ _____

#3 _____ _____

Mean DO conc -- _____ _____

(Set probe to this value while in bottle #4)

Final DO reading-- _____

(The reading in bottle #5 after all the samples are done)

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BOD DETERMINATION

SEED CORRECTION CALCULATION

PLANT: _____ ANALYST: _____ DATE: _____

SAMPLE NO: _____

Seed Dilution	ml Seed	Initial DO (B_1)	5 Day DO (B_2)	% Seed in D_1 / % Seed in B_2 (f)
.3%	.9 ml			
.5%	1.5 ml			
.7%	2.1 ml			

Seed Correction = $(B_1 - B_2)$ f =

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BOD DETERMINATION

BOD CALCULATION

PLANT: _____ ANALYST: _____ DATE: _____

SAMPLE NO: _____ TEST: _____

Seed Correction Factor = $(B_1 - B_2)\ f$ =

BOD (mg/l) = $\dfrac{(D_1 - D_2) - (B_1 - B_2)\ f}{P}$

Volume Sample / Total Volume (P)	Initial DO (D_1)	5 Day DO (D_2)	$\dfrac{(D_1 - D_2) - (B_1 - B_2)\ f}{P}$ = BOD mg/L
Blank			
$\frac{3}{300} = .01$			
$\frac{3}{300}$			
$\frac{3}{300}$			
$\frac{5}{300} = .017$			
$\frac{5}{300}$			
$\frac{5}{300}$			
$\frac{10}{300} = .033$			
$\frac{10}{300}$			
$\frac{10}{300}$			
$\frac{20}{300} = .067$			
$\frac{20}{300}$			
$\frac{20}{300}$			

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TESTING PROCEDURE

T. P. No. 12 Name DISSOLVED OXYGEN ANALYSES, TITRATION METHOD[1]

Date April, 1981 Issue Draft Page 1 of 2

I. INTRODUCTION

Dissolved oxygen (DO) levels in natural and wastewaters are dependent on the physical, chemical and biochemical activities prevailing in the water body. The analysis for DO is a key test in water pollution control and waste treatment process control. DO, at various concentrations, is needed to support aquatic life. This procedure is based on replacing the oxygen in the sample with an equivalent amount of iodine, which is titrated to an end-point.

II. MATERIALS

1. BOD bottles.
2. Titration buret.
3. Widemouth Erlenmeyer Flasks (500 ml).
4. Manganous sulfate solution.
5. Alkali-iodide-azide solution.
6. Polyvinyl alcohol (PVA).
7. Phenylarsine oxide solution (.025N).
8. Concentrated sulfuric acid.
9. Volumetric transfer flasks, polypropylene (203 ml).
10. Automatic pipets (2 ml capacity).

[1]Adapted from: *Standard Methods for the Examination of Water and Wastewater*, 14th Edition, and *Seattle Metro Water Quality Division Procedures Manual*

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III. PROCEDURE

1. Carefully remove the stopper from BOD sample bottle and add 2 ml of manganous sulfate with automatic pipet, well below liquid surface.
2. Add 2 ml of alkali-iodide-azide solution well below liquid surface. Insert stopper, shake and allow precipitate to settle. Shake sample and allow to settle again.
3. Add 2 ml of concentrated sulfuric acid, allowing it to run down inside neck of bottle. Mix well.
4. Pour 203 ml of sample into 500 ml widemouth Erlenmeyer flask.
5. Add 2 to 3 ml of polyvinyl alcohol.
6. Titrate with phenylarsine oxide solution until clear end-point is reached. Record amount (ml) of phenylarsine oxide used.

IV. CALCULATION

DO (mg/1) = Phenylarsine oxide used in titration (ml).

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TESTING PROCEDURE

T. P. No. 13 Name DISSOLVED OXYGEN ANALYSIS, ORION OXYGEN ELECTRODE METHOD[1]

Date April, 1981 Issue Draft Page 1 of 3

I. MATERIALS

1. Orion Model 97-08 oxygen electrode and funnel.
2. pH meter.
3. Magnetic stirrer.
4. BOD bottles, 300 ml.
5. Beaker, 2,000 ml.
6. Compressed air source.
7. Distilled water.
8. Sodium sulfite solution (5%).

II. PREPARATION

1. Set-Up

   a. Connect oxygen electrode to pH meter in the jacks used for pH electrodes.

   b. With electrode switch in "OFF" position and pH meter in pH mode, turn pH controls to 100 percent slope and 25°C. Turn meter calibration knob to obtain 7.00 on the readout.

   c. Turn electrode switch to "BATT" position. The meter readout will be greater than 13 if batteries are all right.

[1]Adapted from: *Seattle Metro Quality Division Procedures Manual* and *Orion Oxygen Electrode Model 97-08 Instruction Manual*

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d. With the electrode switch turned to "ZERO", use the electrode zero control knob to adjust meter reading to 0.00.

2. Testing Response of Electrode

a. Turn electrode switch to H_2O position.

b. Fill a BOD bottle with 5 percent sodium sulfite solution.

c. Place funnel and electrode in bottle, turn on magnetic stirrer and start a timer for 2½ minutes.

d. If meter reads less than .10 mg/1 DO when timer goes off, electrode is working properly.

e. Thoroughly rinse the electrode under cool running tap water.

3. Calibration

a. Aerate a 2,000 ml beaker filled with distilled water.

b. Fill 5 BOD bottles with the aerated water.

c. Titrate 3 of the bottles and calculate the mean dissolved oxygen concentration. (See Dissolved Oxygen Analysis, Titration Method, TP NO. 12).

d. With the electrode switch in the H_2O position, place the probe into the fourth bottle and use the electrode calibration knob to set the meter readout to the mean concentration of the three titrated bottles.

e. Save the fifth BOD bottle (use a water seal) and measure its DO with the probe at the end of the sample run. Record the results.

III. PROCEDURE

1. Dissolved Oxygen

a. Place electrode funnel into DO bottle and seat firmly.

b. Slowly insert electrode into funnel.

c. Turn on magnetic stirrer and stir vigorously. (NOTE: Every sample should be stirred at the same rate).

d. With electrode switch at H_2O position, wait for stable reading.

e. Remove electrode first, and then remove funnel.

f. Rinse electrode and funnel with distilled water and blot dry between readings.

2. Shut-Down

a. Blot probe and funnel dry with Kimwipe.

b. Place electrode and funnel in BOD bottle with enough water to just cover bottom of bottle.

c. Turn electrode switch to "OFF".

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TESTING PROCEDURE

T. P. No. 14 **Name** TOTAL SUSPENDED SOLIDS DETERMINATION[1]

Date April, 1981 **Issue** Draft **Page** 1 **of** 3

I. INTRODUCTION

The composition and concentration of suspended solids in surface waters are important because of their effects of light penetration, temperature, solubility products, and aquatic life. The abrasive action of particulate material affects fish and other aquatic organisms. Suspended particles also transport pesticides, nutrients, metals and other substances that are readily absorbed into or absorbed onto clay particles. This procedure determines total suspended solids (TSS) by filtration.

II. MATERIALS

1. Vacuum filtration assembly.
   a. Glass fiber filter disks, Whatman GF/A, 2.1 cm.
   b. Gooch crucible adaptor, Walter.
   c. Vacuum flask, 1,000 ml.
   d. Gooch crucibles, 22 mm bottom diameter (3 per sample).
   e. Vacuum pump.
   f. Vacuum tubing.
2. Mechanical convection oven.
3. Desiccator.
4. Analytical balance.

[1]Adapted from: *Standard Methods for the Examination of Water and Waste Water*, 14th Edition

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TESTING PROCEDURE

T. P. No. 14 Name TOTAL SUSPENDED SOLIDS DETERMINATION[1]

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5. Pipets, 10 ml.
6. Distilled water.
7. Crucible tongs.

III. PREPARATION

1. Place filter disk into bottom of crucible with wrinkled surface up. Assemble crucible, crucible adaptor, and vacuum flask.
2. Apply vacuum and wash filter with three successive 20 ml volumes of distilled water. Remove all traces of water by vacuum.
3. Remove crucible and dry in mechanical convection oven for 30 minutes.
4. Remove crucibles from oven and place in desiccator. Allow weight to stabilize (approximately 1 hour). Weigh crucibles and record weight.

IV. PROCEDURE

1. Stir sample vigorously and rapidly pipet 30-50 ml sample into crucible (10 ml at a time) and record sample volume. Apply vacuum until all sample has passed through filter.
2. Wash filter with three successive 10 ml volumes of distilled water. Allow complete drainage between washings.
3. Place crucible in oven and dry for 30 minutes at 103-105°.
4. Cool in desiccator until stable. Weigh and record weight.

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 14 **Name** TOTAL SUSPENDED SOLIDS DETERMINATION[1]

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V. CALCULATION

$$\text{Total suspended solids (mg/1)} = \frac{(A - B)\ (1000)\ (1000)}{C}$$

Where A = Weight of crucible, filter and residue (g)

B = Weight of crucible and filter (g)

C = Volume of sample filtered (ml)

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TOTAL SUSPENDED SOLIDS DETERMINATION

PLANT: _____ ANALYST: _____ DATE: _____

SAMPLE NO: _____

$$\text{Total Suspended Solids (mg/l)} = \frac{(A - B)\ (1000)\ (1000)}{C}$$

Where: A = Wt of crucible, filter and residue (g)

B = Wt of crucible and filter (g)

C = Volume of sample filtered (ml)

Crucible No.	Wt. Crucible + filter + residue (g) (A)	Wt. Crucible + filter (g) (B)	Vol. Sample (ml) C	$\frac{A-B\ (1000)(1000)}{C}$ = TSS (mg/l)

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TESTING PROCEDURE

T. P. No. 15 Name OIL AND GREASE DETERMINATION[1]

Date April, 1981 Issue Draft Page 1 of 3

I. INTRODUCTION

This procedure determines total recoverable oil and grease of water and wastewater by serial extraction with Freon.

II. MATERIALS

1. Separatory funnel, 2,000 ml, teflon stopcock, glass stopper.
2. Glass funnel.
3. Filter paper, Whatman Phase Separatory IPS, 11.0 cm.
4. Desicator.
5. Freon. (1, 1, 2 - trichloro - 1, 2, 2 - trifluoroethane).
6. Hydrochloric acid, concentrated.
7. Anhydrous sodium sulfate.
8. Beakers. (150 ml) (1 per sample).
9. Hot plate.
10. Hood.
11. Mechanical convection oven.

III. PROCEDURE

1. If the volume of the sample is unknown, mark the meniscus on the sample bottle for later determination of the volume.
2. If sample is not previously acitified to pH 2 or lower, add 5 ml concentrated hydrochloric acid.

[1]Adapted from: *Standard Methods for the Examination of Water and Waste Water* 14th Edition

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TESTING PROCEDURE

T. P. No. 15 **Name** OIL AND GREASE DETERMINATION[1]

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3. Clean 150 ml beakers are stored in desiccators. Take out one for each sample and weigh them. To clean a beaker, wash it thoroughly with detergent, rinse it with distilled water, wipe down the outside with a Kimwipe soaked with Freon, rinse the inside with Freon, dry it in an oven at 103°C, and store it in a desiccator.
4. Pour the sample into a 2,000 ml separatory funnel.
5. Pour 30 ml of Freon into the sample jar and swirl it around to rinse down the sides.
6. Pour this Freon into the 2,000 ml separatory funnel. Shake it vigorously for 2 minutes, and allow the layers to separate.
7. Drain the separated Freon through Whatman phase separatory filter paper containing 1 gm anhydrous sodium sulfate. Capture the filtered Freon in a tared 150 ml beaker.
8. Repeat steps 4 through 7 two more times, using fresh solvent each time.
9. Combine the extracts in the tared beaker and wash the filter paper with an additional 10-20 ml Freon.
10. Evaporate the Freon from the beaker on a warm (70°C) hot plate under a hood.
11. Dry beakers in an oven at 103°C and cool to a constant weight in a desiccator.

CASTLE & COOKE FOODS

TESTING PROCEDURE

T. P. No. 15 **Name** OIL AND GREASE DETERMINATION[1]

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12. Weigh beaker and record weight.

IV. CALCULATION

$$\text{Oil and grease (mg/l} = \frac{(A - B)\ (1000)\ (1000)}{C}$$

Where A = Weight beaker and residue (g)

B = Weight beaker (g)

C = Volume sample (ml)

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OIL AND GREASE DETERMINATION

PLANT: _____ ANALYST: _____ DATE: _____

SAMPLE NO: _____

Oil & Grease mg/l = $\frac{(A - B)\ (1000)\ (1000)}{C}$

Where A = Wt beaker + residue (g)

B = Wt beaker (g)

C = Volume Sample (ml)

Beaker No.	Wt beaker + residue (g) (A)	Wt beaker (g) (B)	Vol sample ml (C)	$\frac{(A-B)(1000)(1000)}{C}$ = O&G mg/1

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TESTING PROCEDURE

T. P. No. 16 Name DETERMINATION OF NITRITE[1]

Date April, 1981 Issue Draft Page 1 of 2

I. REAGENTS

1. 1% Sulfanilamide solution: 1.0g of sulfanilamide is dissolved in 100 ml of 10% HC1.
2. 0.1% MEDA solution: 0.1g N-(1-Naphthyl)-ethylene diamine dihydrochloride (NEDA) is dissolved in 100 ml nitrite free water.
3. Zinc-uranyl acetate solution: 15g zinc acetate and 5g of uranyl acetate are dissolved in 200 ml water. Shake occasionally and allow to stand overnight and filter.
4. Nitrite standard solution: 5mg of sodium nitrite per ml of water.

II. PROCEDURE

1. Homogenize approximately 100 grams sample in osterizer blender.
2. Weigh 25 gram aliquots of the homogenized sample into 500 ml erlenmeyer flasks.
3. Add about 200 ml nitrite free water and heat to 80°-85°C in a water bath for 30 minutes, with occasional shaking.
4. Cool to room temperature and bring volume to 250 ml with water.
5. To a 20 ml aliquot of the extract, add 5 ml of zinc-uranyl acetate solution to precipitate soluble proteins and mix well.
6. Filter through Whatman No. 42 paper.
7. To 10 ml of the clear filtrate, add 1.0 ml of 1% sulfanilamide solution, let stand for 15 minutes.

[1] Adapted from *Tsuyuki H., etal, Pacific Salmon Roe Processing, Environment Canada Fisheries and Marine Service Technical Report No. 682. 1976.*

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8. Add 1.0 ml of 0.1% NEDA solution, mix well, and let stand for 15 minutes.
9. Read absorbance against a blank at 540 nm in a suitable spectrophotometer.
10. A sodium nitrite standard is carried through the procedure for comparison.

III. JAPANESE PROCEDURE

The following method is used by the Japanese Technicians. It utilizes a kit.

1. Crush sample completely with mortar and pestle.
2. Weigh 4 grams into test tube. Add distilled water to 80 ml total volume.
3. Steam 15 minutes in water bath to coagulate protein.
4. Put test tube in cold water to cool.
5. Add distilled water to 100 ml volume.
6. Shake well.
7. Filter through Whatman No. 42 paper.
8. Add .3-.5 gram G.R. Reagent.
9. Shake well.
10. Let stand 10 minutes for color to develop.
11. Compare sample color to standard ampoules of 3ppm and 5ppm nitrite.